MRID No. 428990-02

DATA EVALUATION RECORD

- 1. CHEMICAL: Oxine Copper. Shaughnessey No. 024002.
- TEST MATERIAL: 1) Quinolate 98; oxine copper or copper 8quinolinolate; Batch No. 52390; 100% active ingredient; a green powder. 2) 14C-oxine copper; Lot No. 029F9233; specific activity of 92.9 μ Ci/mg; 98.25% active ingredient.
- 3. **STUDY TYPE:** 72-1. Freshwater Fish Acute Flow-Through Toxicity Test. Species Tested: Rainbow Trout (Oncorhynchus mykiss).
- CITATION: Ward, G.S. 1993. Oxine Copper (Copper 8-Quinolinolate): Acute Toxicity to Rainbow Trout, Oncorhynchus mykiss, Under Flow-Through Test Conditions. Laboratory Project No. J9006014a. Prepared by Toxikon Environmental Sciences, Jupiter, FL. Submitted by LA QUINOLEINE et ses dérivés, S.A., Paris, France. EPA MRID No. 428990-02.

5. REVIEWED BY:

Mark A. Mossler, M.S. Associate Scientist KBN Engineering and Applied Sciences, Inc.

APPROVED BY: 6.

> Rosemary Graham Mora, M.S. Associate Scientist KBN Engineering and Applied Sciences, Inc.

Henry T. Craven, M.S. Supervisor, EEB/EFED USEPA

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Date: Joseph Aghertur 4/16/95

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Date: 11/22/93

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Date:

Henry T. Craven 2/16/95

- **CONCLUSIONS:** This study is scientifically sound and meets 7. the guideline requirements for an acute toxicity test using rainbow trout. Based on mean measured concentrations, the 96-hour LC_{50} of 8.9 μg ai/l classifies oxine copper as very highly toxic to rainbow trout. The NOEC was $6.2 \mu g$ ai/l.
- 8. RECOMMENDATIONS: N/A.
- 9. **BACKGROUND:**

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

- A. Test Animals: Rainbow trout (Oncorhynchus mykiss) were obtained from a commercial supplier in Red Bluff, CA. The fish were maintained in laboratory freshwater for 40 days prior to test initiation. The temperature during the 7-day period prior to test initiation was 12 ±1°C. Commercial feeds were fed daily during holding. One percent mortality was observed in the population during the 48 hours prior to test initiation. Mean wet weight and standard length of the control fish were 1.0 (0.75-1.6) g and 41 (35-47) mm at the end of the study. Biomass loading rate was approximately 0.12 g/l/day.
- B. Test System: A proportional diluter with a 60% dilution factor was used to prepare the test solutions. The test chambers were 24-1 glass tanks containing 15 l of test solution. The test solution depth was 13 cm. The number of volume additions was approximately 11 per day. The test chambers were randomly positioned in a water bath under a 16-hour light photoperiod with a light intensity of 400-600 lux. Fifteen-minute dawn and dusk simulations were employed.

A stock solution containing 0.7% radiolabeled material and 99.3% unlabeled material was prepared in acidified dimethylformamide (DMF). The concentration of oxine copper in the stock solution was 1 mg active ingredient (ai)/ml. The stock was pumped into the chemical mixing chamber with each diluter cycle providing a nominal concentration of 18 μ g ai/l. This solution was proportionally diluted to provide the remaining treatment solutions.

The test dilution water was treated city water (town of Jupiter, FL). The water was vigorously aerated prior to use. During the test, hardness ranged from 58 to 72 mg/l as $CaCO_3$ and alkalinity ranged from 18 to 24 mg/l as $CaCO_3$. The specific conductivity was 362 μ mhos/cm.

- C. <u>Dosage</u>: Ninety-six-hour, flow-through test. Based on preliminary testing, five nominal concentrations (2.39, 3.98, 6.63, 11.1, and 18.4 μ g ai/l), a solvent control (17 μ l DMF/l), and a dilution water control were prepared for the definitive test.
- D. <u>Design</u>: Twenty trout were impartially distributed (by twos) to each aquarium, one aquarium per treatment or

control. Observations of mortality and sublethal responses were made every 24 hours and dead fish were removed when found. The fish were not fed during testing. The test solutions were aerated throughout the study.

The temperature in the water bath was monitored continuously using a minimum/maximum thermometer and a control tank was monitored hourly using a recording device. The dissolved oxygen concentration (DO) and pH were measured in all test solutions (containing surviving fish) daily.

Water samples from each aquarium were collected at test initiation and termination for analysis of the test material. Concentrations of the test material were measured using liquid scintillation counting.

- E. <u>Statistics</u>: The median lethal concentration (LC₅₀) and associated 95% confidence interval (C.I.) for each 24-hour interval were calculated using a computer program which employed several computational methods.
- 12. REPORTED RESULTS: The mean measured concentrations were 2.34, 3.66, 6.22, 11.3, and 18.3 μ g ai/l (Table 1, attached) and averaged 92 to 102% of nominal.

There was no mortality or sublethal effects in the control or three lowest-concentration treatment groups (Table 2, attached). The 96-hour LC₅₀, based on mean measured concentrations, was 8.94 μ g ai/l (95% C.I.= 6.21-11.3 μ g ai/l) using the binomial probability method. The no-observed-effect concentration (NOEC) was 6.22 μ g ai/l, based on the lack of mortality or sublethal effects at this level.

During the test, the DO ranged from 5.7 to 8.7 mg/l (53 to 80% of saturation) and the pH values ranged from 7.1 to 7.5. The temperature was $11.6-14.7^{\circ}$ C.

13. <u>STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:</u>
No conclusions were made by the author.

Quality Assurance and Good Laboratory Practice (GLP) statements were included in the report, indicating that the study was conducted in accordance with EPA GLP Regulations set forth in FIFRA 40 CFR Part 160.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. <u>Test Procedure</u>: The test procedures were generally in accordance with the SEP, except for the following:

The dilution water was city tap water which was probably chlorinated before being treated in the laboratory. The use of this type water is discouraged. Since no control mortality was observed, the use of this water probably did not affect the results of the study.

The DO of the test solutions fell below 60% of saturation at some points.

The temperature range (11.6-14.7°C) was greater than recommended (12 \pm 1°C).

- B. <u>Statistical Analysis</u>: The reviewer used EPA's Toxanal program to calculate the 96-hour LC₅₀ value and 95% confidence interval (see attached printout) and obtained the same results.
- C. <u>Discussion/Results</u>: Although the DO dropped below 60% of saturation, this only occurred in the dilution water control vessel. There were no mortalities or sublethal effects noted in the vessel, and consequently, the decrease in DO did not affect the outcome of the test. Additionally, the temperature varied by a wide degree, but detrimental effects were not manifested in the controls.

This study is scientifically sound and meets the guideline requirements for an acute toxicity test using rainbow trout. Based on mean measured concentrations, the 96-hour LC₅₀ of 8.9 μ g ai/l classifies oxine copper as very highly toxic to rainbow trout. The NOEC was 6.2 μ g ai/l.

D. Adequacy of the Study:

- (1) Classification: Core.
- (2) Rationale: N/A.
- (3) Repairability: N/A.
- 15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 11-16-93.

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MOSSLER OXINE COPPER ONCORHYNCHUS MYKISS 11-16-93

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
18.3	20	20	100	
11.3	20	18	90	9.536742E-05
6.22	20	0	90	2.012253E-02
3.66	20	^	0	9.536742E-05
2.34	20	0	0	9.536742E-05
	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 6.22 AND 11.3 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 8.94417

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.
